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Production of Polyclonal Antibodies to the Recombinant Coat Protein of *Citrus tristeza virus* and their Effectiveness for Virus Detection

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Abstract

Citrus tristeza virus (CTV) is distributed worldwide and causes the most economically important virus diseases of citrus. Enzyme-linked immunosorbent assay (ELISA) and/or immunoprinting have become an indispensable tools for large-scale diagnosis of CTV worldwide. Several CTV detection kits are commercially available, based on either polyclonal or monoclonal antibodies developed against purified virus preparations. We have developed polyclonal antibodies to recombinant p25 CTV coat proteins (rCP) and determined their effectiveness for both trapping and as the intermediate antibody in double-antibody sandwich indirect (DASI) ELISA. The p25 coat protein gene of three CTV isolates was amplified by RT-PCR and further cloned and expressed in *Escherichia coli* cells. The rCP was injected into rabbits and goats for antibody production. Western blotting assays with the rCP CTV-specific antibodies reacted positively with the homologous and heterologous rCP of the three CTV isolates and with the corresponding native coat protein present in crude sap extracts of CTV-infected citrus tissue, but not with extracts from healthy tissue. The rCP antibodies from goat and rabbit reacted as both plate trapping and intermediate antibodies in DASI-ELISA, discriminating healthy and CTV-infected citrus, with optical density (OD₄₀₅) values in the range of 0.151–2.415 for CTV-infected samples and less than 0.100 for healthy tissue. Commercially available anti-CTV antibodies were used as a reference. Previous reports indicate that antibodies developed to recombinant antigens, including those of CTV, may not be functional for trapping the target antigens under

non-denaturing conditions. Our results showed the feasibility of CTV antibodies developed to the rCP for use as both trapping and intermediate antibodies in DASI-ELISA, when the recombinant antigen was fractionated with polyacrylamide electrophoresis gel and further extensively dialysed against phosphate buffer saline prior to its use as immunogen.

Introduction

Citrus tristeza virus (CTV) is distributed worldwide and causes one of the most economically important virus diseases of citrus (Rocha-Peña et al., 1995a). The virus is transmitted by aphids in a semi-persistent manner, is phloem associated, and has long flexuous particles measuring approximately 2000 × 12 nm (Bar-Joseph and Lee, 1989). CTV has a single-stranded positive sense RNA genome of approximately 19.3 kb (Karasev et al., 1995), and it is encapsidated by two structural coat proteins, a major coat protein (CP) of approximately 26 kDa (Lee et al., 1988) and a minor coat protein of approximately 27 kDa (Febres et al., 1994). The latter covers approximately 5% of the length of the virions at one end (Febres et al., 1996). Within infected tissue, apparent proteolysis of the CP occurs, and frequently produces additional proteins with molecular weights in the range of 21–23 kDa (Lee et al., 1988). The complete genome RNA sequence of several isolates of the CTV RNA has been determined (Karasev et al., 1995; Mawassi et al., 1996; Vives et al., 1999; Yang et al., 1999; Albiachi-Marti et al., 2000).

CTV has occurred in Mexico since the 1980s (Rocha-Peña et al., 2005) and symptomless CTV-infected

trees have been reported in 20 citrus growing states (Silva-Vara et al., 2001; SAGARPA, 2006). Currently, there are approximately 10 certified laboratories nationwide that annually analyse thousands of citrus samples for CTV by ELISA to detect the presence of CTV; for that reason, large amounts of CTV commercial kits are imported every year, mostly from the USA and Spain (Iracheta-Cárdenas et al., 2005). The use of serological methods, mostly the various forms of enzyme-linked immunosorbent assays (ELISA) (Bar-Joseph et al., 1979; Bar-Joseph and Malkinson, 1980; Rocha-Peña et al., 1991a; Garnsey et al., 1993) has become an indispensable tool for large-scale diagnosis of CTV worldwide, for both research and virus control purposes (Bar-Joseph et al., 1979; Garnsey et al., 1981; Bar-Joseph and Lee, 1989; Rocha-Peña and Lee, 1991; Rocha-Peña et al., 1991a,b, 1993; Mathew et al., 1997; Cambra et al., 2000). Antisera production for diagnosis of CTV, either as polyclonal antibodies (Gonsalves et al., 1978; Bar-Joseph and Malkinson, 1980; Marco and Gumpf, 1991) or as monoclonal antibodies (Vela et al., 1986; Permar et al., 1990; Öztürk and Çirakoglu, 2003), was commonly accomplished in the past by using purified virus preparations as immunogens. However, with the sequencing of the CP gene (CPG) from diverse CTV isolates (Sekiya et al., 1991; Mawassi et al., 1993; Pappu et al., 1993a), CTV-specific antisera have been raised against recombinant coat proteins (rCP) expressed in *Escherichia coli* for CTV diagnosis (Manjunath et al., 1993; Nikolaeva et al., 1995; Targon et al., 1997). However, antibodies developed to the expressed recombinant antigens may not be functional for trapping the target antigens under non-denaturing conditions (Nickel et al., 2004). In fact, antibodies produced to the recombinant rCP of CTV, have been reported not to be effective for use as trapping antibodies in double-antibody sandwich indirect (DASI) ELISA protocols (Nikolaeva et al., 1995, 1996). We have characterized several CTV isolates from Mexico (Nava-Coronel et al., 1999; Iracheta-Cárdenas et al., 2002) and have cloned and expressed the CPG in *E. coli* cells (Iracheta-Cárdenas et al., 2002). We report here the production of polyclonal antibodies to the expressed

rCP in *E. coli* and their effectiveness for both trapping and as the intermediate antibody in DASI ELISA.

Materials and Methods

Expression of the recombinant CTV coat protein

The p25 CPG of CTV isolates MX08 and MX14 from Mexico was inserted into the pET22b expression vectors and transformed into *E. coli* BL21 (Iracheta-Cárdenas et al., 2002). Colonies of *E. coli* containing the p25 CPG from CTV isolates MX08 and MX14 (Table 1) were cultured in nutrient broth for 4 h at 37°C, and the expression was induced by adding isopropyl- β -D-thiogalactopyranoside (IPTG). Bacterial cells were then collected by centrifugation and further subjected to subsequent freezing/thawing, sonication and centrifugation treatments for protein extraction (Sambrook et al., 1989). The proteins were then separated by SDS polyacrylamide gel electrophoresis (SDS-PAGE), electro-blotted to nitrocellulose membranes and assayed with polyclonal CTV-specific antibodies (Iracheta-Cárdenas et al., 2002).

Further protein expression and purification

After the expression of the rCP was confirmed for both MX08 and MX14 CTV isolates, the transformed bacterial cells, along with an *E. coli* culture expressing the rCP of the B227 CTV isolate from India (Table 1; Manjunath et al., 1993) were further cultured in nutrient broth, and proteins were separated as previously described (Iracheta-Cárdenas et al., 2002). The proteins from bacterial cell isolates were separated in polyacrylamide-resolving gels by discontinuous electrophoresis. The protein bands of approximately 27 kDa were visualized with 0.1% Coomassie Blue and excised, then immediately subjected to a second electrophoretic separation as indicated above. The second set of gels were then stained, destained and the protein bands of approximately 27 kDa were excised, and extensively dialysed against phosphate saline buffer (PBS = 8 mM Na₂HPO₄, 14 mM KH₂PO₄, 15 mM NaCl, pH 7.4). Extensive dialysis consisted of four to five changes of 5 l buffer, 12 h each at 5°C, until SDS was completely removed. After dialysis, the polyacryl-

Table 1

Properties of *Citrus tristeza virus* (CTV) isolates used for developing antibodies to recombinant coat protein and evaluating them as antigens in serological test for CTV detection

CTV isolate	Origin	Strain type	MCA 13 reaction	References
MX08	Nuevo León, México	Mild isolate, type T30 from Florida	–	Iracheta-Cárdenas et al., 2002; Silva-Vara et al., 2001; Rocha-Peña, unpublished
MX14	Tamaulipas, México	Severe isolate, similar to B247 stem pitting in sweet orange from Venezuela	+	Iracheta-Cárdenas et al., 2002; Silva-Vara et al., 2001; Rocha-Peña, unpublished
B227	India	Severe isolate, stem pitting in grapefruit, Coorg and Cleopatra mandarins, and rough lemon	+	Manjunath et al., 1993
T36	Florida, USA	Severe inducing decline type isolate	+	Garnsey et al., 1987
T55	Florida, USA	Mild isolate in sweet on sour orange combinations	–	Rocha-Peña et al., 1992, 1995b
T66	Florida, USA	Severe inducing decline type isolate	+	Rocha-Peña et al., 1992, 1995b

amide gel portions containing the rCP protein from every treatment were individually packed as 1 ml doses in 3 ml disposable syringes and stored at -20°C until used for immunization.

Immunization protocols

Four doses of the SDS-PAGE purified proteins were separately injected into rabbits at 15 day intervals. The first dose was injected subcutaneously and consisted of approximately 100 μg of protein mixed with 1 ml of Freund's complete adjuvant. Two subsequent intramuscular injections included 100 μg of protein mixed with 1.0 ml of Freund's incomplete adjuvant. The fourth injection was given without adjuvant 7 days after the third injection. The animals were bled prior to start of immunization protocol and 1 week after the fourth injection, by nicking the marginal ear and heart puncture, respectively. Antisera to CTV purified rCP preparations were also developed in goats by four subcutaneous and intramuscular injections following a protocol as described for the rabbits, but the doses of antigen consisted of approximately 200 μg of protein. The goats were bled from the jugular vein before the immunization protocol started and 1 week after the fourth injection. The blood samples from both animal species were incubated for 2 h at 37°C , and then kept at 5°C for 18 h. The antiserum from every sample was recovered by centrifugation, and kept in a freezer at -20°C . Table 2 shows the origin of the different antibodies developed.

Western blotting

The rCP proteins from the transformed bacterial cells for MX08, MX14 and B227 CTV isolates were dissociated and electrophoresed on SDS-PAGE as previously described (Iracheta-Cárdenas et al., 2002). A culture of untransformed *E. coli* BL21 was included as a negative control. The gels were electrotransferred to nitrocellulose membranes (Schleicher & Schuell, 0.45 μ pore) for 1 h by using a Semi-Dry blotter (EBU-4000, CA, USA). After electroblotting, the membranes were soaked for 30 min in Tris buffered saline-Tween blocking solution [(TBS = 0.02 M Tris, 0.5 M NaCl pH 7.5) plus 0.05% Tween 20] and washed twice with 25 ml TBS, for 2 min each. Afterwards, the membranes were incubated for 1 h with the corresponding rCP CTV-specific antiserum (dilution 1 : 10 000), then washed twice with TBS-Tween (TBS plus 0.5 ml Tween 20/l) and then individually incubated for 1 h with a commercial anti-species immunoglobulins (IgG)

conjugated with alkaline phosphatase. Goat anti-rabbit IgG conjugate (Sigma A 8025, St. Louis, MO, USA) at a dilution of 1 : 1000 was used for those antisera developed in rabbits, whereas rabbit anti-goat IgG conjugate (Sigma A4187) at a dilution of 1 : 1000 was used for those antisera developed in goats. After washing with TBS-Tween as before, the sites of antigen localization were visualized by incubating the membranes for 5–10 min in 5 ml of a mixture of nitro blue tetrazolium (NBT) and 5-bromo-4-chloro-3-indoyl phosphate (BCIP; Pierce 34042, Rockford, IL, USA). The colour reaction was stopped by transferring the membranes to distilled water.

Evaluation

The relative sensitivity of each anti-rCP antiserum was compared for its ability to detect CTV by DAS-ELISA (Bar-Joseph and Malkinson, 1980; Clark et al., 1986). Commercially available anti-CTV antibodies were used as a reference. IgG from each anti-rCP antiserum were purified by ammonium sulphate precipitation and adjusted to 1.0 mg/ml ($\text{OD}_{280} = 1.45$) by spectrophotometry (Clark et al., 1986). In each experiment (Table 3), CTV antigens included two replications of citrus extracts (1 : 10 w/v) infected with T36, T55 and T66 CTV isolates (Table 1). An extract of healthy citrus was used as the negative control. Likewise, each experiment included two replications of CTV antigens using the complete CTV kit from Agdia Inc. (Elkhart, IN, USA). In Experiment 1, goat anti-CTV antibodies from Agdia were used as trapping antibody; IgG of rabbit anti-rCP were evaluated as intermediate antibodies at 0.20, 0.10, 0.050 and 0.025 $\mu\text{g}/\text{ml}$; enzyme conjugate was goat anti-rabbit IgG (Sigma A8025). In Experiment 2, rabbit anti-CTV antibodies from Bioreba (Reinach BL, Switzerland), were used as trapping antibody; IgG of goat anti-rCP were evaluated as intermediate antibodies; enzyme conjugate was rabbit anti-goat IgG (Sigma A4187). In Experiments 3 and 4, respectively, goat and rabbit anti-rCP immunoglobulins were evaluated separately as trapping antibodies, at dilutions of 1–3 $\mu\text{g}/\text{ml}$. The rest of the test was performed using Agdia's intermediate antibodies and enzyme conjugates (Table 3). In the four experiments, IgG anti CTV from Agdia and Bioreba were used at concentration recommended by the suppliers. Unless otherwise stated, 100 μl was used per well and three washings with PBS-Tween [phosphate-buffered saline (8 mM Na_2HPO_4 , 14 mM KH_2PO_4 , 15 mM NaCl, pH 7.4), plus 0.05% Tween 20] were performed between steps. Polystyrene flat bottom Nunc Maxisorp (Rochester, NY, USA) microtiter plates were coated with the corresponding trapping antibodies diluted in carbonate buffer (0.015 M NaHCO_3 , 0.03 M NaCO_3 , pH 9.6), and incubated for 6 h at 37°C . Citrus samples were homogenized using an IKA Ultra Turrax T25 Basic Tissue mixer (Wilmington, NC, USA) at 1 : 10 (w/v) in extraction buffer [PBS-Tween 20, 2% (w/v) polyvinylpyrrolidone (Sigma PVP-40)], and added to the wells and incubated for 18 h at 5°C .

Table 2
List of antibodies developed against the recombinant p25 coat protein of *Citrus tristeza virus* (CTV)

Antibody ID	CTV isolate	Immunized animal
C3	MX08	Rabbit
C7	MX14	Rabbit
C9	B227	Rabbit
T1	MX14	Goat

Table 3
Combinations of *Citrus tristeza virus* (CTV) specific antibodies from commercial sources and from animals immunized with the recombinant coat protein (rCP) from CTV isolates MX08 (rabbit C3), MX14 (rabbit C7, and goat T1) and B227 (rabbit C9), evaluated in double-antibody sandwich indirect enzyme-linked immunosorbent assay recorded for Experiments 1, 2, 3 and 4 (see Table 1 for details)

Experiment. no.	Trapping antibody	Intermediate antibody	rCP and commercial antibody combinations OD ₄₀₅ values for antigens ^a				Reference Agdia commercial ELISA CTV Kit OD ₄₀₅ values for antigens ^a					
			T36	T55	T66	Range	Healthy	T36	T55	T66	Range	Healthy
1	Goat anti-CTV (Agdia)	Rabbit anti-rCP C3	0.336 (0.009)	1.131 (0.038)	1.650 (0.258)	0.336–1.650	0.049 (0.011)	2.333 (0.2467)	0.545 (0.161)	2.149 (0.533)	0.545–2.333	0.053 (0.000)
1	Goat anti-CTV (Agdia)	Rabbit anti-rCP C7	2.056 (0.231)	0.335 (0.062)	1.595 (0.044)	0.335–2.056	0.036 (0.009)	0.855 (0.303)	0.573 (0.415)	1.498 (1.046)	0.573–1.498	0.033 (0.009)
1	Goat anti-CTV (Agdia)	Rabbit anti-rCP C9	2.415 (0.083)	1.298 (0.232)	2.446 (0.038)	1.298–2.415	0.095 (0.006)	2.320 (0.148)	2.190 (0.028)	2.320 (0.141)	2.190–2.395	0.049 (0.012)
2	Rabbit anti-CTV (Bioreba)	Goat anti-rCP T1 ^d	0.489 (0.062)	0.319 (0.118)	0.913 (0.085)	0.319–0.913	0.038 (0.021)	2.011 (0.469)	1.1615 (0.577)	1.983 (0.516)	1.615–2.011	0.037 (0.022)
3	Goat anti-rCP (T1)	Mouse anti-CTV (Agdia)	0.418 (0.099)	0.583 (0.042)	1.116 (0.393)	0.418–1.116	0.084 (0.012)	2.018 (0.681)	0.602 (0.130)	2.307 (0.272)	0.602–2.307	0.041 (0.008)
4	Rabbit anti-rCP (C3)	Mouse anti-CTV (Agdia)	2.294 (0.291)	0.393 (0.019)	2.091 (0.181)	0.393–2.294	0.082 (0.006)	2.391 (0.153)	2.352 (0.097)	2.578 (0.242)	2.352–2.578	0.017 (0.019)
4	Rabbit anti-rCP (C7)	Mouse anti-CTV (Agdia)	1.599 (0.498)	0.145 (0.030)	1.279 (0.036)	0.145–1.599	0.024 (0.004)	0.787 (0.284)	0.642 (0.226)	0.765 (0.253)	0.642–0.787	0.032 (0.027)
4	Rabbit anti-rCP (C9)	Mouse anti-CTV (Agdia)	0.562 (0.089)	0.151 (0.016)	0.824 (0.02)	0.151–0.824	0.053 (0.062)	1.417 (0.082)	1.043 (0.101)	1.981 (0.057)	1.043–1.981	0.040 (0.053)

^aMean of two replications in every test. Number in parenthesis indicates SD values.

Each of the intermediate antibodies were diluted in conjugate buffer [(PBS–Tween PVP) plus 0.2% bovine serum albumin (w/v)], added to the appropriate plates and incubated for 4 h at 37°C. After washing, alkaline phosphatase enzyme conjugates were diluted in conjugate buffer and incubated for 2 h at 37°C. At the end of the incubations, the reaction with 1.0 mg/ml *p*-nitrophenyl phosphate (Sigma, N 2765) in 10% triethanolamine, pH 9.8, was quantified after 1 h at 405 nm (OD₄₀₅) using a BioTek EL311 ELISA plate reader (Winooski, VT, USA).

Results

Expression of the CTV rCP

Polyacrylamide gel electrophoresis of the p25 CPG of CTV isolates expressed in *E. coli*, as well as crude sap of healthy and CTV-infected citrus tissue is shown in Fig. 1a. Transformed *E. coli* cultures showed the rCP as a prominent protein band of approximately 26 kDa for MX08, around 28 kDa for MX14 and approximately 26 kDa for B227 CTV isolates, respectively (Fig. 1a, lanes 2, 3 and 4). No evidence of such rCP was observed in untransformed culture of *E. coli* BL21 included as a negative control (Fig. 1a lane 1). Crude sap, from healthy tissue (Fig. 1a, lane 5) and for CTV-infected tissue (Fig. 1a, lanes 6, 7, 8 and 9) showed uniform protein profiles of diverse molecular sizes.

Reactivity of antibodies to rCP under denaturing conditions

Western blotting assays with all the antibodies developed from the CTV rCP showed a positive reaction with the homologous and heterologous rCP of the three CTV isolates and with the corresponding native CP present in crude sap of CTV-infected citrus tissue (Fig. 1b). The rabbit anti-rCP antibodies (ID code C3) developed to MX08 CTV isolate (Fig. 1b) produced a strong positive reaction with the rCP of the three bacterial cultures transformed with MX08, MX14 and B227 CTV isolates, respectively (Fig. 1b, lanes 2, 3, and 4). Likewise, there was a positive serological reaction with proteins of approximately 26–28 kDa present in the citrus tissue infected with the MX08, MX14, T66 and T36 CTV isolates, used as positive controls (Fig. 1b, lanes 6, 7, 8 and 9). No serological reaction was observed with either the bacterial proteins of the untransformed *E. coli* BL21 cells, or crude sap from healthy citrus (Fig. 1b, lanes 1 and 5). Whereas, antibodies produced in rabbits to the rCP of MX14 (ID code C7) and B227 (ID code C9) CTV isolates and in goats for MX14 (ID code T1) (Fig. 1b; Table 1) provided comparable positive reactivity to the rCP from transformed *E. coli* cells and CTV-infected citrus tissue; they did not react with untransformed *E. coli* BL21 cells, nor crude sap from healthy citrus (Fig. 1b). No serological reaction was observed in similar Western blotting assays conducted with normal sera before immunization from any of the animal species (data not shown).

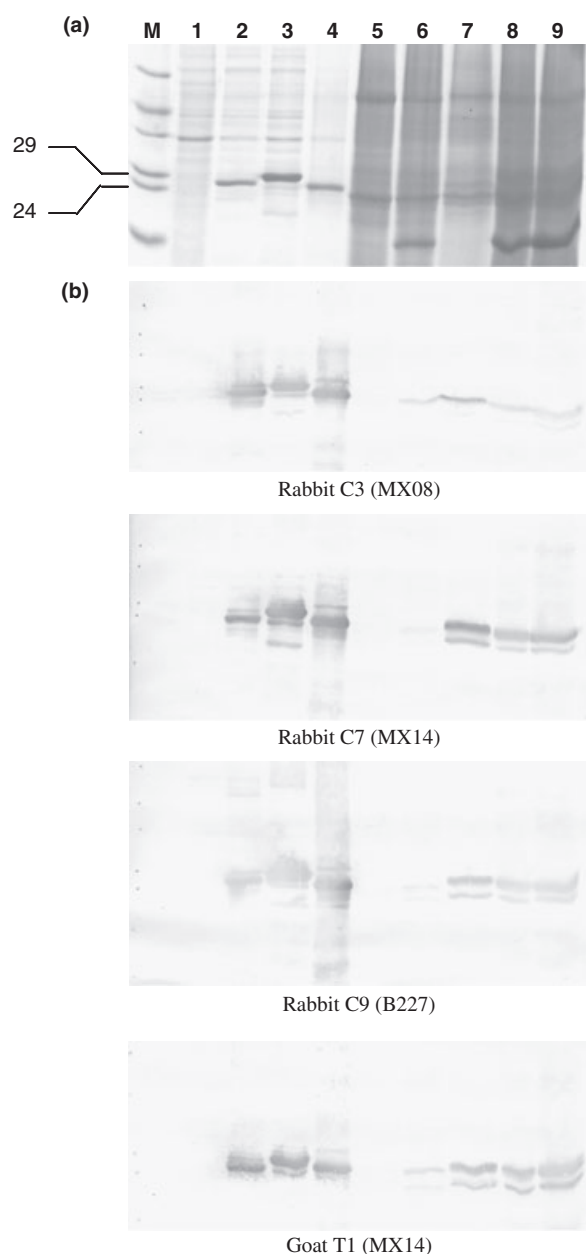


Fig. 1 Western blot analysis of the recombinant p25 coat protein (rCP) of several *Citrus tristeza virus* (CTV) isolates expressed in *Escherichia coli*. (a) Polyacrylamide 12% gel electrophoresis. M, molecular-weight markers (kDa); lane 1, bacterial culture of *E. coli* without transformation (negative control). Lanes 2, 3, and 4 bacterial cultures of *E. coli* transformed with the B227, MX08 and MX14 CTV isolates, respectively. Lane 5, healthy citrus (negative control). Lanes 6, 7, 8 and 9, citrus tissue infected with MX08, MX14, T66 and T36 CTV isolates, respectively. (b) Immunodetection with rCP antibodies developed against CTV isolates

Reactivity of antibodies to rCP under non-denaturing conditions

The reactivity of rabbit and goat antibodies to CTV rCP was evaluated by DAS-ELISA (Bar-Joseph and Malkinson, 1980) using commercial CTV antibodies as a reference (Table 3). In Experiment 1, all rabbit CTV rCP antibodies, used as intermediate antibodies gave a positive reaction to all CTV antigens tested

(Table 3). Rabbit C3 antibodies developed against MX08 CTV isolate gave OD_{405} values in the range of 0.336–1.650 for CTV antigens (Table 3). Rabbit C7 and C9 antibodies, developed against MX14 and B227 CTV isolates, respectively, gave OD_{405} values in the range of 0.335–2.056 and 1.298–2.415 for CTV antigens, respectively. In Experiment 2, goat T1 anti-rCP were used as intermediate antibodies, and gave OD_{405} values in the range of 0.319–0.913 for CTV antigens (Table 3). In Experiment 3, goat CTV rCP antibodies were used as trapping antibodies, and gave a positive reaction to all CTV antigens tested with OD_{405} values in the range of 0.418–1.116 (Table 3). Rabbit C3, C7 and C9 CTV rCP were used in Experiment 4, as trapping antibodies, and gave OD_{405} values in the range of 0.393–2.294, 0.145–1.599 and 0.151–0.824, respectively for CTV antigens (Table 3). For all rCP antibody combinations, OD_{405} values were in the range of 0.024–0.095 for crude sap from healthy plants used as a negative control (Table 3). The commercial kit from Agdia was used as a reference, and gave a positive reaction for all three CTV antigens with OD_{405} values in the range of 0.545–2.578; likewise, the OD_{405} values with crude sap from healthy plants were in the range of 0.017–0.053 (Table 3).

Discussion

In this study, several antibodies were produced to the CTV rCP and further evaluated under denaturing and non-denaturing conditions for specificity against the rCP and native CTV antigens. Western blot analysis showed comparable positive reactivity of the rCP antibodies from the three different CTV isolates and two different animal species (rabbit and goat) to the homologous and heterologous rCP of the three CTV isolates and with the native CP present in crude sap of CTV-infected citrus tissue (Fig. 1b–e). These results showed the ability of the rCP antibodies to efficiently detect the rCP and CTV antigens under denaturing conditions (Fig. 1a). Likewise, rCP antibodies showed comparable reactivity as trapping and intermediate antibodies under non-denaturing conditions in DAS-ELISA (Table 3). The differences in reactivity among the rCP from rabbit and goat prepared to MX08, MX14 and B227 CTV isolates, as expressed as OD_{405} values with CTV antigens (Table 3), may be due to differences in immunological response in the injected animals, rather than differences among CTV isolates.

The use of rCP as an immunogen to develop antibodies has been of great value particularly for viruses that are difficult to purify (Petrovic et al., 2003) and for those that are subject to degradation during the purification process (Barbieri et al., 2003). It has been reported that antibodies developed to expressed recombinant antigens may not be functional for trapping the target antigens under non-denaturing conditions, such as in DAS-ELISA (Nickel et al., 2004). In this regard, there are reports that antibodies produced to the rCP of CTV are not

effective for use as trapping antibodies in double-antibody sandwich ELISA protocols (Nikolaeva et al., 1995, 1996). It was suggested that the rCP expressed in *E. coli* apparently did not fold as the native CP (Nikolaeva et al., 1996). To overcome this apparent deficiency, Bar-Joseph et al. (1997) proposed to use booster injections with partially purified CTV particles after initial immunizations of the animal with rCP antigens, to enhance virus trapping to the ELISA plates. In our study, where the immunization protocol included three injections of 100 µg of the rCP and a fourth (booster) application also with 100 µg of the rCP, the antibodies produced were capable of trapping non-denatured CTV on ELISA plates. The antibodies reported here can be used effectively as capture antibodies in DASi-ELISA protocols. This was clearly demonstrated by the resulting OD₄₀₅ values in Experiment 3 that used goat rCP antibodies, 0.418–1.116, and in Experiment 4 that used rabbit rCP antibodies, 0.218–1.752 (Table 3).

The specific methods used in this study can explain this. The rCPs expressed in *E. coli* and further fractionated by SDS-PAGE, were extensively dialysed against PBS prior to immunization. This might have facilitated the removal of SDS from the acrylamide and aided the rCP recovered to regain some of its native folding. Another possibility is that the antibodies produced to the rCP from MX08, MX14 and B227 CTV isolates, are specific for epitopes present in the primary structure of the protein which are not susceptible to SDS denaturing. This has been reported for antibodies developed to the capsid and p24 proteins of *Beet yellows virus* (Agranovski et al., 1994) and the MCA13 (Pappu et al., 1993b) and 3DF1 (Pappu et al., 1995) monoclonal antibodies developed against CTV. There are some other reports on the production of polyclonal antibodies developed to recombinant antigens of *Prunus dwarf virus* (Abou-Jawdah et al., 2004), *Grapevine leaf roll associated virus-3* (Ling et al., 2000) and *Tomato spotted wilt virus* (Vaira et al., 1996).

The Central California Tristeza Eradication Agency (CCTEA) (Mathew et al., 1997; Polek, 2000; Gottwald et al., 2002) routinely uses rCP antibodies (Nikolaeva et al., 1995) for large-scale CTV detection; however, it is limited in its use as the intermediate antibody in the DASi-ELISA. The CCTEA uses goat anti-CTV antibodies developed against purified virus preparations by conventional methods for trapping (M.L. Polek, personal communication 2004). No other large-scale programme reports the use of rCP antibodies for CTV detection. We have developed antibodies raised against CTV rCPs that have trapping properties in DASi-ELISA and which can discriminate positive and negative CTV-infected samples (Table 3). These findings enable their use for routine large-scale indexing of CTV. This is supported by Sandoval-Alejos et al., 2006 who used a combination of goat T1 for trapping and rabbit C3

as the intermediate anti rCP CTV antibodies. This combination accurately discriminated the positive and negative CTV-infected citrus samples and was comparable to those obtained using commercial CTV detection ELISA kits.

The availability of the antibodies developed against the rCP of CTV isolates MX08, MX14 and B227 and their potential for commercialization, is considered a viable option for large-scale CTV indexing, along with the other commercial serological kits for CTV detection available mostly from USA and Spain (Sandoval-Alejos et al., 2006). In addition, it could prevent to some extent the dependency of Mexico's national campaign for CTV detection (SAGARPA 2004) from importing commercial CTV kits from overseas (Sandoval-Alejos et al., 2006).

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